Structural Studies of a Yeast Quaternary Transcription Factor Complex

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Abstract No. wang9077
Beamline(s): **X25**

Introduction: The key step in eukaryotic transcription initiation is the recruitment and assembly of dozens of proteins around a DNA promoter to form a pre-initiation complex (PIC). The core of the PIC is a 'quaternary' assembly consisting of TATA-box DNA, TATA-box binding protein (TBP), and transcription factors IIA (TFIIA) and IIB (TFIIB). These factors combine to delineate the start site of transcription, specify the orientation of transcription, and promote the maintenance of transcription in the presence of transcriptional repressors. The key to understanding the organization and stability of the 'quaternary complex' is to elucidate its three-dimensional structure. In particular, we seek to understand the stereochemical basis for the interactions of TFIIB and TFIIA with each other and with DNA sequences that flank the 8 basepair TATA box sequence.

Methods and Materials: We have crystallized a yeast quaternary transcription factor complex consisting of TATA-box DNA, yeast TBPc, yeast TFIIAΔ113, and yeast TFIIBc. The crystals grow from PEG 4000 precipitants as tetragonal pyramids and are typically 200 x 200 x 100 um. Data collection was performed at NSLS Beamline X25. Typical quaternary complex crystals diffract to about 8 Å.

Results: The crystals belong to spacegroup P42(1)2, with unit cell dimensions a = b = 149 Å, c = 110 Å. This is consistent with one 98 kD quaternary complex per asymmetric unit, and a solvent content of 68%. We have been able to grow significantly larger crystals of the complex recently (600 x 400 x 200 um), and a 3.6 Å data set was collected from these crystals at NSLS Beamline X25. A molecular replacement solution is in progress.

Conclusions: Good diffraction from quaternary complex crystals depends on having large crystals, low mosaicity, and high brilliance synchrotron radiation.